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# Voltage-dependent Conductances in *Limulus* Ventral Photoreceptors

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**ABSTRACT** The voltage-dependent conductances of *Limulus* ventral photoreceptors have been investigated using a voltage-clamp technique. Depolarization in the dark induces inward and outward currents. The inward current is reduced by removing  $\text{Na}^+$  or  $\text{Ca}^{2+}$  and is abolished by removing both ions. These results suggest that both  $\text{Na}^+$  and  $\text{Ca}^{2+}$  carry voltage-dependent inward current. Inward current is insensitive to tetrodotoxin but is blocked by external  $\text{Ni}^{2+}$ . The outward current has a large transient component that is followed by a smaller maintained component. Intracellular tetraethylammonium preferentially reduces the maintained component, and extracellular 4-amino pyridine preferentially reduces the transient component. Neither component is strongly affected by removal of extracellular  $\text{Ca}^{2+}$  or by intracellular injection of EGTA. It is concluded that the photoreceptors contain at least three separate voltage-dependent conductances: 1) a conductance giving rise to inward currents; 2) a delayed rectifier giving rise to maintained outward  $\text{K}^+$  current; and 3) a rapidly inactivating  $\text{K}^+$  conductance similar to the A current of molluscan neurons.

## INTRODUCTION

The receptor potential in *Limulus* ventral photoreceptors is initiated by the entry of  $\text{Na}^+$  ions through a light-sensitive conductance in the plasma membrane (Millecchia and Mauro, 1969 *a* and *b*). The waveform of the receptor potential is governed in part by the kinetics of this conductance, and also in part by voltage-dependent conductances. In a previous study, Pepose and Lisman (1978) showed that depolarization of the membrane in the dark produces a complex combination of inward and outward current and that the steady-state outward current is due primarily to a voltage-activated  $\text{K}^+$  conductance termed the delayed rectifier. In the experiments reported here, we have used pharmacological and ion substitution techniques to characterize the membrane conductances responsible for the other currents evoked by membrane depolarization. Some of these results have been presented previously in abstract form (Lisman et al., 1979; Fain et al., 1979) and in a review (Fain and Lisman, 1981).

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## METHODS

Voltage-clamping and recording methods were similar to those described in Lisman and Brown (1971) with the exception that the membrane current was compensated for leakage under the assumptions that the leakage conductance was independent of voltage and that the membrane current in the range of  $-70$  to  $-90$  mV was due entirely to leakage current (see Pepose and Lisman, 1978). Leakage compensation was obtained by subtracting a signal proportional to command voltage from the total current. The proportionality constant was adjusted so that a 20-mV hyperpolarizing voltage-clamp pulse from a holding potential of  $-70$  mV produced no current deflection (other than the capacitive transients). Membrane current was measured with a current-to-voltage transducer of time constant 1 ms; the interval between voltage-clamp pulses was  $\sim 10$  s. In some experiments, we injected tetraethylammonium (TEA) or ethyleneglycol-*bis*-( $\beta$ -amino-ethyl ether) *N,N*-tetra-acetic acid (EGTA). In these experiments the cell was impaled with either one double-barreled and one single-barreled electrode, or three independent electrodes. TEA electrodes contained 0.1 M TEA, and EGTA electrodes contained 0.3 M EGTA, brought to neutral pH with KOH. A bucking current of 1–2 nA served to prevent leakage of these substances into the cell. The other two electrodes were used to voltage-clamp the cell during the injection, so that the injection itself would not produce any change in membrane potential. The composition of solutions is given in Table I. 4-Aminopyridine (4-AP) was obtained from ICN Pharmaceuticals; TEA was obtained from Eastman Kodak Co., Rochester, N. Y. To facilitate impalement, the preparation was treated with 20 mg/ml Pronase (Calbiochem-Behring Corp., San Diego, Calif.) for 1 min.

Before proceeding to a description of membrane currents, it is necessary to discuss whether the voltage-clamp technique typically used by us and others to study ventral photoreceptors with axons gives an accurate measure of the voltage-dependent conductances in the cell soma. Since ventral photoreceptors have a long ( $\sim 1$  cm) axon that terminates in the brain (Clark et al., 1969), and since this axon is not likely to be isopotential over its whole length, axonal currents may distort the currents that flow during voltage-clamp pulses. It has been shown in molluscan neurons that axotomy has very obvious effects on the voltage-clamp currents (Connor, 1977). Thus, to study the influence of the axon on *Limulus* ventral photoreceptors, we cut ventral nerves into small (600  $\mu$ m) pieces, each containing a single photoreceptor. The distance between the junction of the axon and the soma and the cut end of the axon varied from 100  $\mu$ m to 400  $\mu$ m in the 13 axotomized cells studied. The currents in Fig. 1 are from one of these preparations and are representative of 11 of 13 axotomized cells. In the other two the transient outward current was almost completely absent for reasons that remain unclear. The currents in all other figures included in this paper were obtained from cells with long axons typical of the ventral nerve preparation used by ourselves and others (e.g., Millecchia and Mauro, 1969 *a* and *b*). Since the qualitative features of the current are the same as those in Fig. 1, we conclude that the axon does not seriously distort voltage-dependent current.

## RESULTS

The voltage-activated membrane current of the dark-adapted photoreceptor was examined by giving depolarizing voltage-clamp pulses from a holding potential of  $-70$  mV (Fig 1). This current was compensated for leakage current, as described in Methods. 10-mV depolarizing pulses evoked no inward or outward current. Larger depolarizing pulses (to potentials between  $-50$

and  $-10$  mV) evoked a net inward current with a maximum value of 10–20 nA, which reached a peak 5–20 ms after the onset of the pulse (for better resolution of inward current, see Fig 2 A and B). The net current observed at these potentials remained inward for only a short interval and became outward within  $<100$  ms. Depolarizing pulses to potentials more positive than  $-10$  mV, evoked net outward current at all times. The fact that the *net* current was outward does not preclude the possibility that inward current was present but was masked by coincident outward current. A striking feature of the outward current in the voltage range between  $-20$  and  $+30$  mV is its transient nature. The outward current decayed (in  $\sim 1$  s) to a nearly constant maintained value. We shall refer to the current measured at the end of 1–2 s pulses as “steady state,” although in some cases the current had not quite reached a steady level. The transient current at  $+30$  mV was about twice the magnitude of the steady-state outward current in the cell illustrated in Fig. 1 A and B. This ratio varied from 1.5 to 3 among cells.

TABLE I  
COMPOSITION OF SOLUTIONS

	KCl	NaCl	MgCl <sub>2</sub>	MgSO <sub>4</sub>	Tris-Cl	CaCl <sub>2</sub>	Choline-Cl	NiCl <sub>2</sub>	CdCl <sub>2</sub>	CoCl <sub>2</sub>	EGTA
	<i>mM</i>						<i>mM</i>			<i>mM</i>	
ASW	10	425	22	26	15	10	—	—	—	—	—
0-Ca SW	10	425	32	26	15	—	—	—	—	—	—
0-Ca EGTA SW	10	425	32	26	15	—	—	—	—	—	0.5
EGTA-SW	10	425	22	26	15	11	—	—	—	—	1.0
0-Na SW	10	—	22	26	440	10	—	—	—	—	—
0-Na SW (choline)	10	—	22	26	15	10	425	—	—	—	—
0-Na, 0-Ca SW	10	—	32	26	440	—	—	—	—	—	—
Ni-SW	10	425	12	26	15	10	—	10	—	—	—
Cd-SW	10	425	12	26	15	10	—	—	10	—	—
Co-SW	10	425	12	26	15	10	—	—	—	10	—

pH of solutions was 7.8 except those in which NaCl had been replaced with Tris-Cl. In these cases the pH was 7.0, as were the pH of the control solutions.

#### *Ionic Basis of Inward Currents*

Voltage-dependent inward current carried by  $\text{Na}^+$  and  $\text{Ca}^{2+}$  has been demonstrated in a variety of vertebrate and invertebrate neurons (see Hagiwara, 1975). To examine the ionic basis of the inward current in *Limulus*, we removed  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ , or both from the extracellular solution. Replacement of  $\text{Na}^+$  by Tris (0-Na SW) reduced but did not abolish inward current (Fig. 2 A). For five cells studied, the average reduction of inward current was  $62 \pm 13\%$  (SD). The remaining inward current was nearly eliminated by removing  $\text{Ca}^{2+}$  (0-Na, 0-Ca SW). When  $\text{Ca}^{2+}$  alone was removed from seawater (0-Ca SW), the inward current was somewhat reduced (Fig. 2 A); for six cells, the average reduction in inward current was  $35 \pm 13\%$  SD. The remaining inward current was subsequently abolished by removing  $\text{Na}^+$  (0-Na, 0-Ca SW). Similar results were obtained using choline as a  $\text{Na}^+$  substitute. Since removal of  $\text{Na}^+$  or  $\text{Ca}^{2+}$  alone only partially reduced the net inward current, whereas the removal of both ions practically abolished it, we conclude that both ions carry inward current. The inward current that remains after  $\text{Na}^+$  removal ( $\text{Ca}^{2+}$  current)

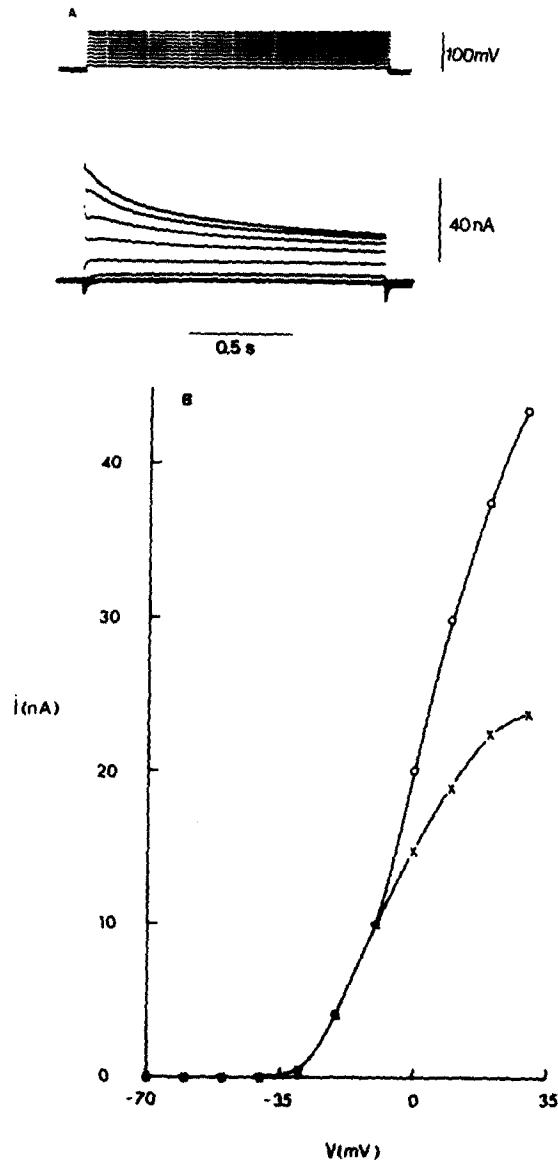


FIGURE 1. Membrane current during voltage-clamp pulses in the dark. (A) Voltage pulses were given in 10-mV increments from a holding potential ( $V_H$ ) of -70 mV. Outward currents for voltages from -60 to -10 mV are small, and at this magnification appear hidden in the noise. The first voltage to show an easily observable outward current is -20 mV. The currents then rise steeply as voltage is increased up to +30 mV. (B) The total currents ( $i$ ) measured at 100 ms (O) and 1.5 s (X) after the beginning of the pulse are plotted vs. membrane potential ( $V$ ). The data in this figure were taken from an axotomized cell.

and that which remains after  $\text{Ca}^{2+}$  removal ( $\text{Na}^+$  current) have approximately the same time-course but different voltage dependences (Fig. 2 B). The  $\text{Na}^+$  current appeared to be activated at voltages more negative than those that activated the  $\text{Ca}^{2+}$  current, though the apparent voltage dependences may

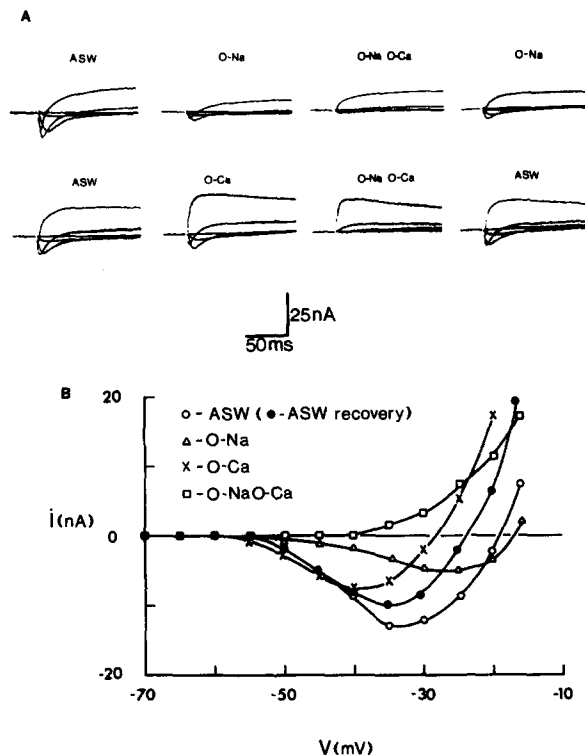


FIGURE 2. Reduction of early inward currents in  $\text{Na}^+$ -free and  $\text{Ca}^{2+}$ -free seawater. (A) Voltage-clamp pulses were presented in 10-mV increments to a maximum of  $-20$  mV ( $V_H = -70$  mV). The resulting currents in ASW consisted of an early inward phase followed by an outward phase. Early inward currents were reduced by removal of  $\text{Na}^+$  and nearly eliminated by subsequent removal of  $\text{Ca}^{2+}$ . The effect was largely reversible. When  $\text{Ca}^{2+}$  was removed from the seawater, the inward currents were reduced somewhat, and subsequent removal of  $\text{Na}^+$  eliminated the remaining inward current. (B) Early currents (measured 8 ms after the beginning of the pulse) are plotted vs. the membrane potential ( $V$ ). Early currents in ASW, 0-Na SW, and 0-Ca SW showed somewhat different voltage dependences.

have been influenced by changes in surface potential or contributions from outward currents.

Most voltage-dependent channels specific for  $\text{Na}^+$  are blocked by tetrodotoxin (TTX), and Dowling (1968) showed that spikes recorded in the eccentric

cells of *Limulus* are blocked by  $6.2 \times 10^{-8}$  M TTX. In contrast, the spikes seen in the retinula cells of the lateral eye (Dowling, 1968) and in *Limulus* ventral photoreceptors (Bayer and Barlow, 1978) are not blocked by TTX. We have examined the effects of TTX on the inward currents in ventral photoreceptors. Since in some other preparations (Lee et al., 1977) the TTX sensitivity of  $\text{Na}^+$  channels can be removed by treatment with protease of the sort we and others routinely use to aid impalement of *Limulus* photoreceptors (see Methods), we tested for the effects of TTX with and without enzymatic pretreatment. In neither case did TTX have any effect at concentrations up to  $3 \times 10^{-6}$  M.  $10 \text{ mM Ni}^{2+}$ , on the other hand, blocked the inward currents completely (Fig. 3).  $\text{Ni}^{2+}$  has been shown to reduce both  $\text{Na}^+$  (Hille et al., 1975) and  $\text{Ca}^{2+}$  currents (Akaike et al., 1978) in other systems. Partial blockage

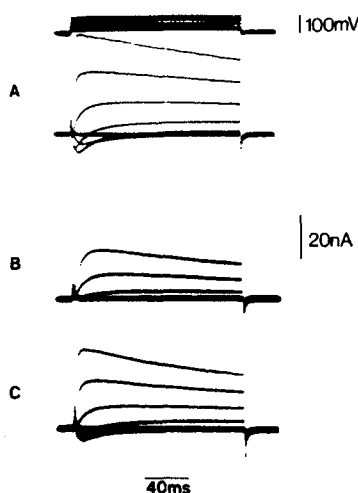


FIGURE 3. Block of inward currents by extracellular  $\text{Ni}^{2+}$ . Application of  $10 \text{ mM}$  extracellular  $\text{Ni}^{2+}$  completely eliminated early inward currents elicited by voltage-clamp pulses ( $V_H = -70 \text{ mV}$ ). The effect was partially reversible. A: ASW; B: NiSW; C: ASW recovery.

(~75%) of the inward currents was obtained with  $10 \text{ mM Co}^{2+}$ . Very little blockage (<5%) was obtained with  $10 \text{ mM Cd}^{2+}$ . We have not examined whether these partial effects are the result of preferential suppression of  $\text{Ca}^{2+}$  or  $\text{Na}^+$  currents. Inward currents could also be reduced or abolished by the organic blockers verapamil ( $100 \mu\text{M}$ ) and D-600 ( $1 \text{ mM}$ ).

#### *Pharmacological Separation of Outward Currents*

The transient nature of the outward current (Fig. 1 A) could be explained by a partial inactivation of a single conductance (Aldrich et al., 1979), or by the presence of two or more conductances with different time-courses. In molluscan neurons there are several pharmacologically distinct types of  $\text{K}^+$  channels. One of these rapidly inactivates during maintained depolarization with a time constant of the order of a few hundred milliseconds (Hagiwara et al., 1961;

Connor and Stevens, 1971 *b*; Neher, 1971). We shall refer to this current as  $i_A$ , and its channel as the A channel (Adams et al., 1980).  $i_A$  can be observed only if the holding potential ( $V_H$ ) is below  $-40$  mV; at  $V_H$  above  $-40$  mV, the A channel is almost completely inactivated. A second  $K^+$  channel, termed the delayed rectifier, carries a maintained outward current ( $i_K$ ) and shows less inactivation (Connor and Stevens, 1971 *a*).

There is suggestive evidence from previous work that the outward currents in *Limulus* also consist of more than one component. Pepose and Lisman (1978) showed that in *Limulus* the transient nature of the outward current during large depolarizing pulses depends strongly on holding potential in the way one would expect if a current similar to  $i_A$  were present. That is, the transient current is absent at a  $V_H$  of  $-30$  mV or above (see right half of Fig. 6 A and B) and increases gradually as  $V_H$  is made more negative. This suggests that the total outward current arises from two conductances, one that gives rise to a maintained outward current and one that undergoes complete inactivation, as found in molluscan neurons.

To examine this question further, we have looked for pharmacological agents that affect the two phases of outward current differently. Fig 4 A shows the inward and outward currents during voltage-clamp pulses before and after intracellular injection of TEA (see Methods). TEA almost completely blocked the maintained outward current (Fig. 4 A and B). The early outward current (100 ms), however, was much less affected (Fig. 4 A and C). The amount of TEA required to block the maintained outward current can be estimated from Fig. 5 A and B in which the steady-state outward current before TEA injection and after successive injections is plotted; each injection raised the intracellular TEA concentration by  $\sim 0.2$  mM. A half-maximal block was produced by a TEA concentration of  $\sim 0.5$  mM, a value comparable to that which blocks the voltage-dependent  $K^+$  channel in squid giant axon (Armstrong and Binstock, 1965). Very large injections ( $>100$  mM TEA) blocked the early outward currents as well as the steady-state currents. The results therefore suggest that there are two outward currents, a maintained outward current that is preferentially blocked by intracellular TEA, and a transient outward current that is much less sensitive to TEA. We shall refer to the transient outward current in *Limulus* as  $i_A$  because it strongly resembles the A current of mollusks in the voltage dependence and time constant of its inactivation (see below). We shall refer to the steady-state current as  $i_{ss}$ .

Thompson (1977) has shown in snail neurons that  $i_A$  is preferentially blocked by 4-amino-pyridine (4-AP). Fig. 6 shows that this is also the case in *Limulus*. The addition of  $0.3$  mM 4-AP blocked the transient component of outward current elicited by depolarizing voltage pulses from a  $V_H$  of  $-70$  mV, but had much less effect on the maintained outward current (Fig. 6 A). When voltage-clamp pulses were given from a  $V_H = -30$  mV, 4-AP had relatively little effect (Fig. 6 B), presumably because the transient outward current had already been inactivated at this  $V_H$ . Fig. 6 C is a plot of the maintained outward current as a function of voltage. 4-AP clearly affects the voltage dependence of the maintained current, but these effects are small compared



with the large reduction in the transient outward current, which has been plotted in Fig. 6 D according to the procedure described in the next paragraph.

The voltage and time dependence of transient outward current ( $i_A$ ) can be approximated by making the simplifying approximations that the maintained current reaches a constant value within 50 ms (see Fig. 6, left side), and that

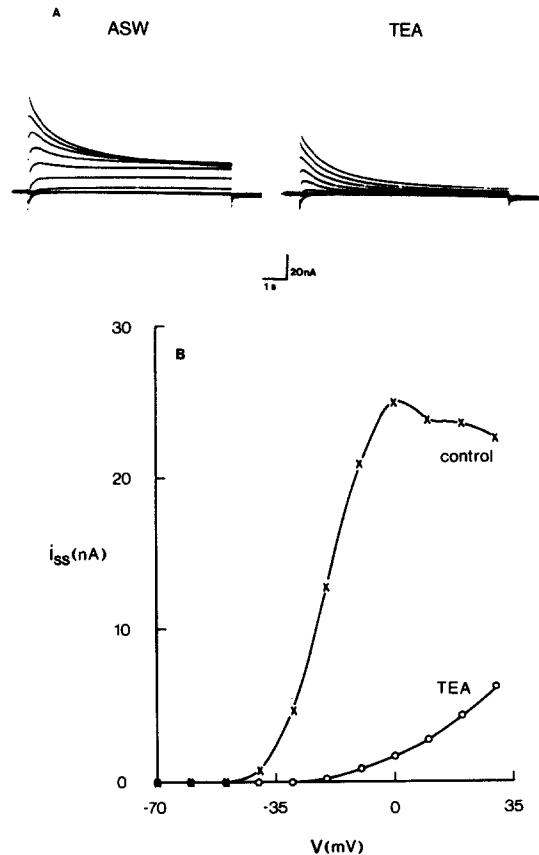


FIGURE 4. Effect of intracellular injection of TEA on voltage-dependent currents. (A) TEA drastically reduced the maintained outward current elicited by voltage-clamp pulses ( $V_H = -70$  mV). Currents are for voltage pulses in 10-mV increments from  $-60$  to  $+30$  mV. (B) The maintained outward currents ( $i_{ss}$ ) measured 2 s after the beginning of the pulse are plotted vs. membrane potential ( $V$ ) for control and TEA-injected conditions. (C) The early outward currents measured 100 ms after the beginning of the pulse are plotted vs. membrane potential ( $V$ ).

transient outward current decays to 0 by the end of 1.4-s voltage-clamp pulses (see Fig. 4 A). In this case  $i_A(t) = i(t) - i_{ss}$  for times  $> 50$  ms. The time dependence of  $i_A$  computed in this way is shown in Fig. 7A. The decay of outward current was approximately exponential both before and after TEA injection, although the fit was considerably better after TEA injection. The

time constant of exponential decay ( $\tau$ ) before and after TEA injection agreed to within 20% and was not strongly voltage dependent in either case, increasing at most by 25% for a 50-mV increase in depolarizing pulse amplitude in TEA-injected cells. The average  $\tau$  at +30 mV was  $365 \pm 110$  ms SD ( $n = 6$  cells). The voltage dependence of  $i_A$  before and after TEA injection is shown in Fig. 7 B. This plot reveals that the transient outward current is only weakly affected by TEA injection. In subsequent figures the inactivating outward current,  $i_A$ , is plotted, rather than the total early current  $i(t)$ , which includes both activating and inactivating currents.

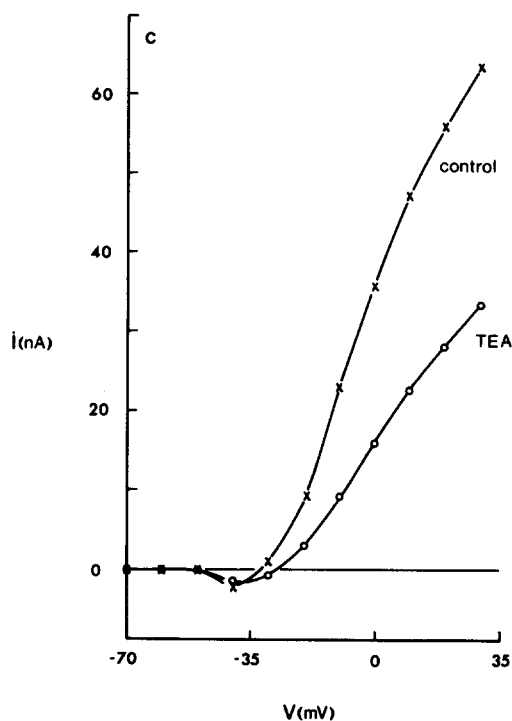


FIGURE 4.

In addition to the maintained and transient outward currents, there appears to be a maintained voltage-dependent current not blocked by large injections of TEA (Figs. 4 B and 5 B). This current appears to be similar to the voltage-dependent leakage current found in snail neurons after blockage of  $K^+$  currents with  $Cs^+$  (Kostyuk and Krishtal 1977; Akaike et al., 1978).

#### *Dependence of Outward Currents on $Ca^{2+}$ Entry*

The classic voltage-dependent  $K^+$  channel, as originally described by Hodgkin and Huxley (1952), is gated directly by voltage. More recently, a qualitatively different kind of  $K^+$  channel gated by a rise in  $Ca_i^{2+}$  has been described (Meech and Standen, 1975). Many neurons appear to have both kinds of  $K^+$

channels. The component of outward current carried by  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels can be blocked by removing  $\text{Ca}_o^{2+}$ , by pharmacologically blocking  $\text{Ca}^{2+}$  channels, or by buffering  $\text{Ca}_i$  with EGTA (Meech, 1978).

In *Limulus*, the removal of extracellular  $\text{Ca}^{2+}$  had relatively little effect on outward current (Fig. 8 A–C). This was the pattern observed in 6 of the 10 cells studied. In the other four, the outward current in 0- $\text{Ca}^{2+}$ -SW had a more complex relationship to the control currents; at voltages below zero the outward currents were slightly greater than in ASW (see Fig. 2 A, lower traces), but at more positive voltages the outward currents were 5–10% less

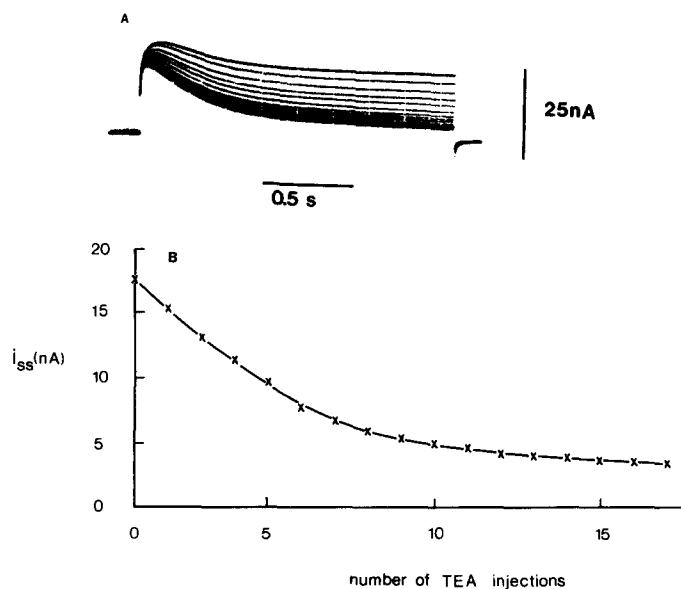


FIGURE 5. Development of TEA block of outward current. (A) Responses to successive 60-mV depolarizing voltage-clamp pulses (1/10 s) are superposed ( $V_H = -70$  mV). Each pulse was followed by an injection of TEA, each of which was  $4 \times 10^{-8}$  C. Thus, assuming a transport coefficient of 0.26 (Nicholson et al., 1979) and a cell volume of  $5 \times 10^{-10}$  liter, each injection raised  $\text{TEA}_i \sim 0.2$  mM. (B) The maintained currents,  $i_{ss}$ , are plotted vs. TEA injection number. A half-maximal block of  $i_{ss}$  was achieved after about three injections. The early current was less affected.

than in ASW. To ensure that  $\text{Ca}^{2+}$  is removed from the extracellular space and the deep invaginations of the plasma membrane, it is common practice to add the  $\text{Ca}^{2+}$ -sequestering agent EGTA to low- $\text{Ca}^{2+}$  solutions. Fig. 8 A–C shows that the addition of 0.5 mM EGTA had no greater effect on outward currents than simply removing  $\text{Ca}^{2+}$  from the seawater. These experiments indicate that if *Limulus* ventral photoreceptors contain a  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channel, this channel must carry a small fraction of the total outward current.

The experiments shown in Fig. 8 A–C were conducted in Waltham, Mass. Similar experiments performed in Woods Hole, Mass. gave equivalent results when  $\text{Ca}^{2+}$  was simply removed from the external solutions. However, when

EGTA was added to 0- $\text{Ca}^{2+}$  SW, the experiments in Woods Hole showed a large (60%) depression of maintained outward current. The transient outward current was much less affected. As a control for a pharmacological action of EGTA, we used a solution that contained 1 mM EGTA and 11 mM  $\text{Ca}^{2+}$ . Thus this solution contained 1 mM of EGTA complexed with  $\text{Ca}^{2+}$  and a free- $\text{Ca}^{2+}$  concentration equal to that of normal ASW. The solution produced a depression of maintained outward current similar to that of the 0-Ca EGTA solution. We therefore concluded that the depression of outward current was an effect of EGTA itself (Fain et al., 1979). In Woods Hole, this effect was consistently and repeatedly observed (12 separate trials) using several different sources of EGTA. In Waltham, on the other hand, this EGTA effect was not observed in four separate trials, using EGTA from one of the same bottles used in Woods Hole. The reason for this discrepancy is still unclear.

Another way to study the dependence of outward current on  $\text{Ca}^{2+}$  entry is to block  $\text{Ca}^{2+}$  entry pharmacologically using 10 mM  $\text{Ni}^{2+}$  (see Fig. 3). Fig. 9 A-C shows that both transient and maintained currents were reduced in amplitude after addition of  $\text{Ni}^{2+}$ . The current-voltage curves in Fig. 9 B and C show that  $\text{Ni}^{2+}$  somewhat decreased the maximum current and shifted the  $i$ - $V$  curves to the right. These effects of  $\text{Ni}^{2+}$  may have resulted from a change in surface potential or a direct effect of  $\text{Ni}^{2+}$  on the conductance of the delayed rectifier rather than from a blockage of inward  $\text{Ca}^{2+}$  current (see Discussion), and thus do not necessarily indicate that a component of outward current is  $\text{Ca}^{2+}$  activated.

Intracellular injection of EGTA provides yet another test for a  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  conductance. Fig. 10 A shows the voltage-dependent currents before and after iontophoretic injection of EGTA. The inward current, transient outward current, and maintained current were not strongly affected, but a small (~25%) decrease in maintained outward current was consistently observed (Fig. 10 B and C). To test whether the injected EGTA actually stabilized  $\text{Ca}_i$ , we monitored the waveform of the response evoked by long-duration light. Normally, such responses have a large initial transient that decays to a plateau. Direct measurements of  $\text{Ca}_i$  with aequorin show that EGTA blocks the light-induced changes of  $\text{Ca}_i$ . When this occurs, the decline from the transient phase to plateau phase of the receptor potential is blocked (Brown and Blinks, 1974). Fig. 10 D shows that the EGTA injection in the experiment of Fig. 10 A was sufficient to block the decline of the light-induced current from transient to plateau and thus was probably enough to buffer  $\text{Ca}_i^{2+}$ . The reduction of voltage-dependent outward current by EGTA could imply that a small component of outward current is  $\text{Ca}^{2+}$  activated, but it is also possible that intracellular EGTA directly reduces the voltage-dependent  $\text{K}^+$  conductance, as reported by Kostyuk and Krishtal (1977), in molluscan neurons.

#### DISCUSSION

In this paper we have used pharmacological and ion substitution techniques in order to characterize the voltage-dependent conductances in the membrane of *Limulus* ventral photoreceptors. Our aim is to provide a clearer understand-

ing of the various components of the voltage response evoked by light (O'Day et al., 1982). We have shown that *Limulus* photoreceptors have at least three separate voltage-dependent conductances. During depolarizing voltage pulses,

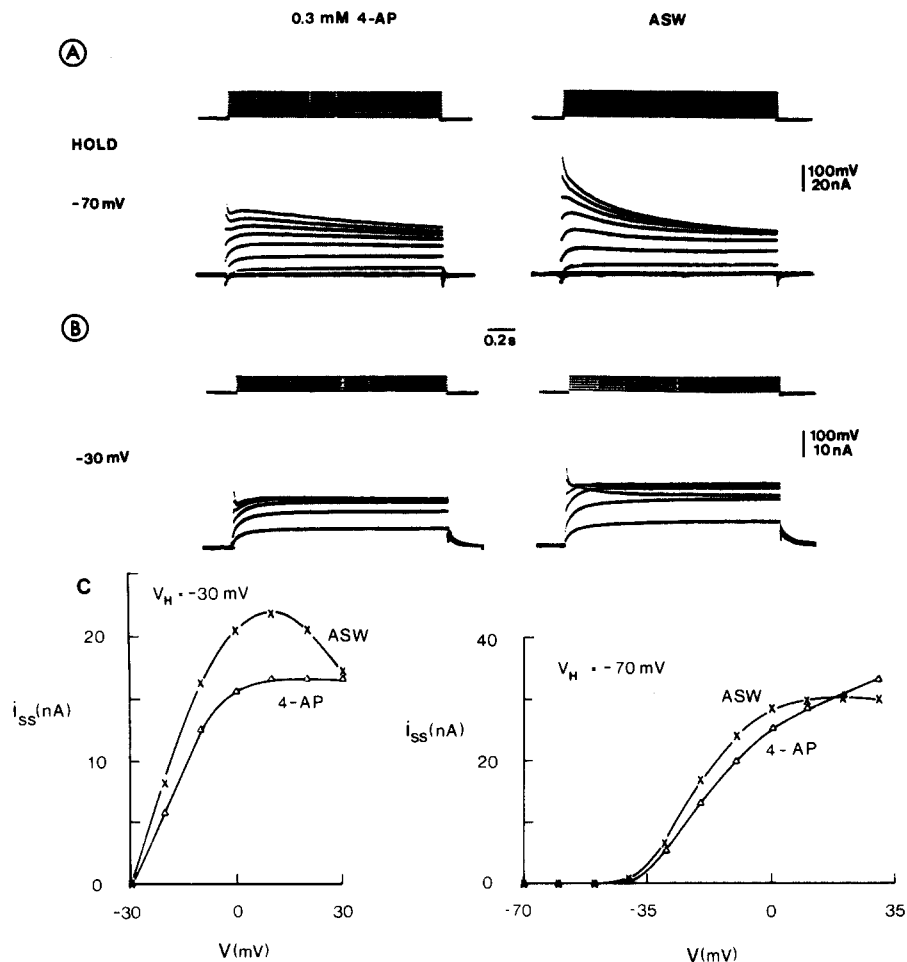


FIGURE 6. Effects of 4-AP on voltage-dependent currents. (A) Voltage-clamp pulses were presented during application of 0.3 mM 4-AP and in ASW recovery. When holding potential,  $V_H$ , was  $-30$  mV, 4-AP had only a small effect on the voltage-dependent currents. (B) When the holding potential was  $-70$  mV, the effect of 4-AP was a large reduction of the transient outward currents, whereas the maintained currents were much less affected. (C) The maintained currents,  $i_{ss}$ , are plotted vs. the membrane potential ( $V$ ) in 4-AP and ASW for the two different  $V_H$  values. (D)  $i_A$ , the difference between early current ( $t = 100$  ms) and  $i_{ss}$ , is plotted vs.  $V$ .

these conductances produce an inward current carried by  $\text{Ca}^{2+}$  and  $\text{Na}^+$ , a transient outward current resembling the A current of molluscan neurons, and a sustained outward current similar to the delayed rectifier of the squid giant axon. We shall discuss the properties of each of these conductances below.

*Inward Current*

The evidence of Fig. 2 strongly suggests that both  $\text{Na}^+$  and  $\text{Ca}^{2+}$  carry inward current. Because there is as yet no pharmacological or kinetic evidence for the separation of the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  currents, it is possible that both ions pass through the same channel. However, this has not been shown to occur in any other system of which we are aware (see Hagiwara and Byerly, 1981). It seems more likely that  $\text{Na}^+$  and  $\text{Ca}^{2+}$  enter through separate channels. The data in Fig. 2 B suggest that the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  currents have different voltage dependence; that is, the inward current in  $0\text{-Ca}^{2+}$  SW is activated at less

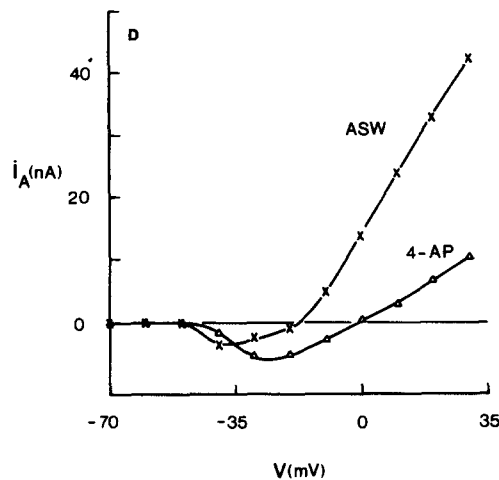


FIGURE 6.

positive voltages than that in  $0\text{-Na}^+$ . This has also been observed in molluscan neurons (Adams et al., 1980) and tunicate eggs (Okamoto et al., 1976 *a* and *b*), where the evidence for separate  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channels is compelling. If the  $\text{Na}^+$  current in *Limulus* is carried through a separate  $\text{Na}^+$  channel, it is a channel very different from the one that gives rise to most axonal spikes in at least three ways: first, the inward  $\text{Na}^+$  current in *Limulus* activates more slowly (Fig. 2 A and B) than axonal  $\text{Na}^+$  current, for example in squid axon (Hodgkin and Huxley, 1952); second, the inward current is not blocked by TTX at concentrations that block axonal spikes in squid axon and in *Limulus* lateral eye (Dowling, 1968); third, the  $\text{Na}^+$  current is blocked by  $\text{Ni}^{2+}$  at a concentration that reduces, but does not block,  $\text{Na}^+$  currents in axons (Hille et al., 1975). Further work is required to define more clearly the conductances responsible for inward current.

Another aspect of the inward current in *Limulus* that remains to be studied is its inactivation. On the basis of the work reported here it is unclear whether the inward current rapidly inactivates during maintained depolarization or whether there is a non-inactivating component. O'Day et al. (1982) have studied the desensitization of the light-induced current produced by  $\text{Ca}^{2+}$  entry during voltage-clamp pulses. They have found that the amount of desensitization increases as the duration of the depolarizing pulse is increased

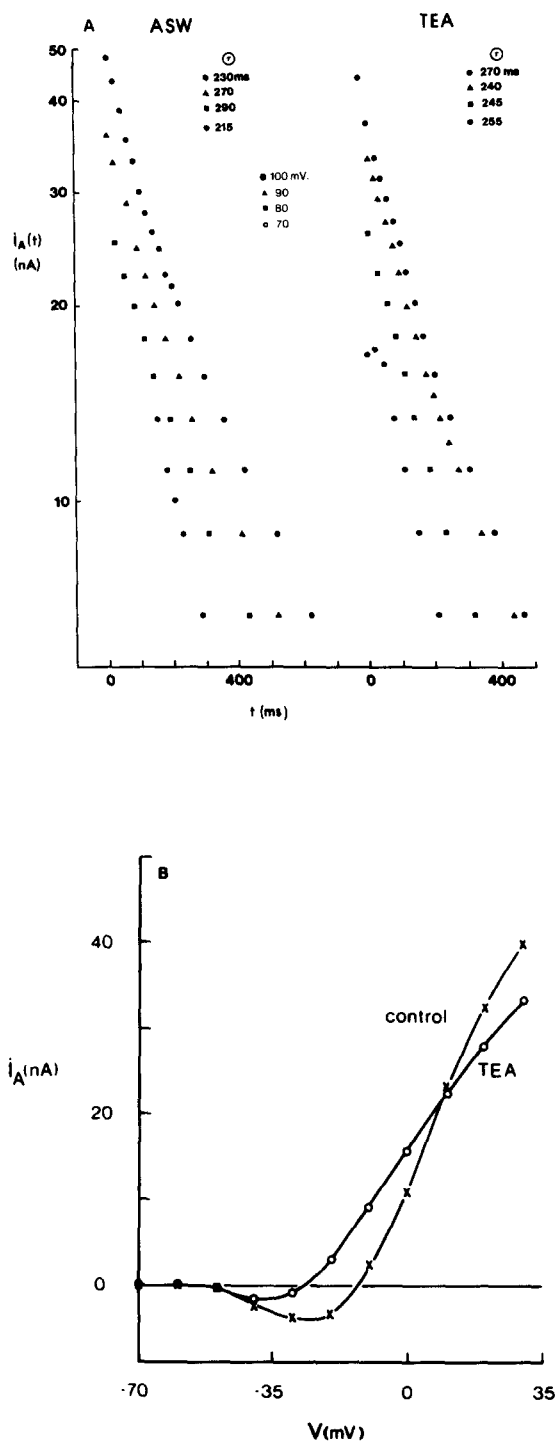


FIGURE 7.

up to at least 10 s, a result that suggests that the  $\text{Ca}^{2+}$  conductance does not completely inactivate, even during very long depolarizations.

#### *Transient Outward Current*

Our experiments show that the outward current evoked by depolarizing voltage pulses consists of at least two components. One of these is a transient or rapidly inactivating current ( $i_A$ ) that is preferentially blocked by 4-AP and is relatively insensitive to intracellular TEA. From records in which the maintained component of outward current has been blocked by TEA (Fig. 5), we can deduce that activation is rapid, with the current reaching its peak within  $<10$  ms.  $i_A$  is activated at voltages more positive than  $-20$  mV and inactivates with an exponential time-course having a time constant of  $\sim 350$  ms at  $+30$  mV. Because the transient outward current becomes progressively inactivated as the holding potential is made more positive than  $-60$  mV (Pepose and Lisman, 1978), the state of inactivation of this conductance in the dark depends strongly on the value of resting potential, which can vary in different preparations from  $-70$  to  $-40$  mV.

The properties of the transient outward current in *Limulus* are very similar to transient outward currents in other preparations (Adams et al., 1980). The A currents in both mollusks and *Limulus* decline exponentially with time constants of several hundred milliseconds; both have a voltage dependence such that small changes in resting potential have a large effect on the state of inactivation, and both are blocked by low concentrations of 4-AP. However, the transient outward current in mollusks appears to be more sensitive to intracellular TEA than that in *Limulus* (Neher and Lux, 1972). Furthermore, in *Limulus* the transient and steady-state currents are activated in the same voltage range, whereas in molluscan neurons the transient current can be activated at voltages more negative than those required to activate the steady-state current (Hagiwara et al., 1961; Connor and Stevens, 1971 b).

The transient outward current in *Limulus* is directly voltage gated, rather than dependent on entry of extracellular  $\text{Ca}^{2+}$ . The evidence for this is that the transient outward current persists in  $\text{Ca}^{2+}$ -free seawater and when  $\text{Ca}^{2+}$  entry is blocked by  $\text{Ni}^{2+}$ . It is also unaffected by intracellular injection of EGTA. In molluscan neurons, the transient outward current is also unaffected by changes in internal  $\text{Ca}^{2+}$  (Connor, 1979). A transient outward current that is  $\text{Ca}^{2+}$  dependent has been reported in uterine smooth muscle, certain crustacean muscle, and vertebrate heart muscle (Vassort, 1975; Mounier and

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FIGURE 7. Voltage and time dependence of transient outward current. (A)  $i_A$  is plotted vs. time on a semi-logarithmic scale. Each curve is labeled with the amplitude of the voltage pulse in millivolts. The decay of  $i_A$  was roughly exponential in control conditions and more closely exponential after TEA injection. Time constants of  $i_A$  decay ( $\tau$ ) are listed. (B) The value  $i_A$ , the total current [ $i(t)$ ] minus the maintained current ( $i_{ss}$ ), is plotted vs. membrane potential ( $V$ ) for  $t = 100$  ms.  $i_A$  was less affected by TEA injection than the total early current or  $i_{ss}$ . Computed from data in Fig. 4 A.



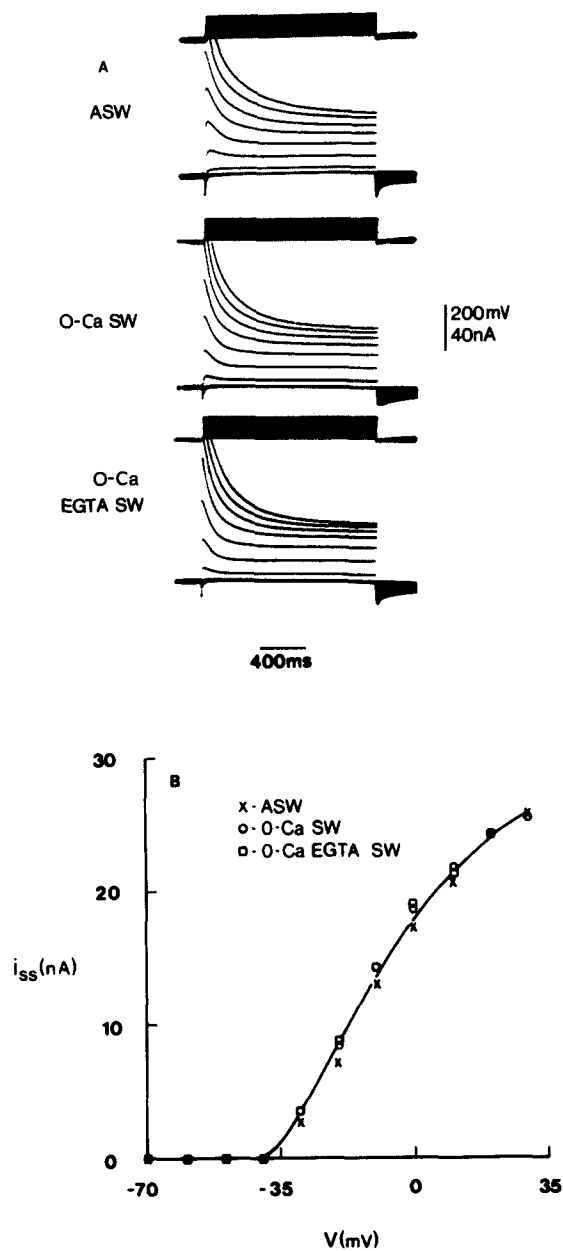


FIGURE 8. Effects of removal of extracellular  $\text{Ca}^{2+}$  on outward current. (A) Removal of  $\text{Ca}^{2+}$  had negligible effects on the maintained and transient outward currents elicited by voltage-clamp pulses ( $V_H = -70$  mV). (B) The voltage dependence of  $i_{ss}$  is shown for ASW (x), 0-Ca SW (o), and 0-Ca EGTA SW (□). (C) The voltage dependence of  $i_A$  is shown for ASW (x), 0-Ca SW (o), and 0-Ca EGTA SW (□).

Vassort, 1975; Sieglebaum and Tsien, 1979). The transient outward current in *Limulus* is blocked by low concentrations of 4-AP and high concentrations of TEA. Since both drugs are classic  $K^+$  channel blockers, the transient outward current is likely to be carried by  $K^+$ .

#### *Maintained Outward Current*

The evidence that the maintained outward currents are carried by voltage-sensitive  $K^+$  channels was presented by Pepose and Lisman (1978). The principle reason for re-examining the maintained currents in this paper is a recent suggestion by Schmidt and Fein (1979) that ventral photoreceptors

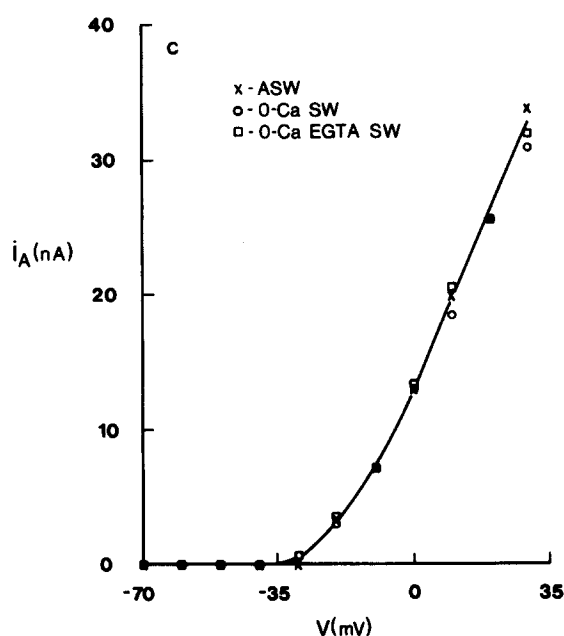


FIGURE 8.

may contain a  $Ca^{2+}$ -activated  $K^+$  channel. They found that putative blockers of inward  $Ca^{2+}$  current produced a depression of voltage-dependent outward current. Consistent with their hypothesis of a  $Ca^{2+}$ -activated  $K^+$  channel, we have found that there is a voltage-dependent inward  $Ca^{2+}$  current, and that when this current is blocked by  $Ni^{2+}$ , the maintained outward current is reduced.

It is possible, however, to explain this reduction without assuming that outward currents depend on inward currents. In Fig. 9 B it can be seen that a major effect of  $Ni^{2+}$  was to shift the current-voltage curve to more positive voltages. Shifts of this sort have been observed in other preparations and have been shown to arise from changes in surface potential that are probably

caused by the binding of  $\text{Ni}^{2+}$  to the outer membrane surface (see, for example, Hille et al., 1975). Notice that  $\text{Ni}^{2+}$  causes the current-voltage curves of both  $i_K$  and  $i_A$  to shift along the voltage axis by approximately the same amount.

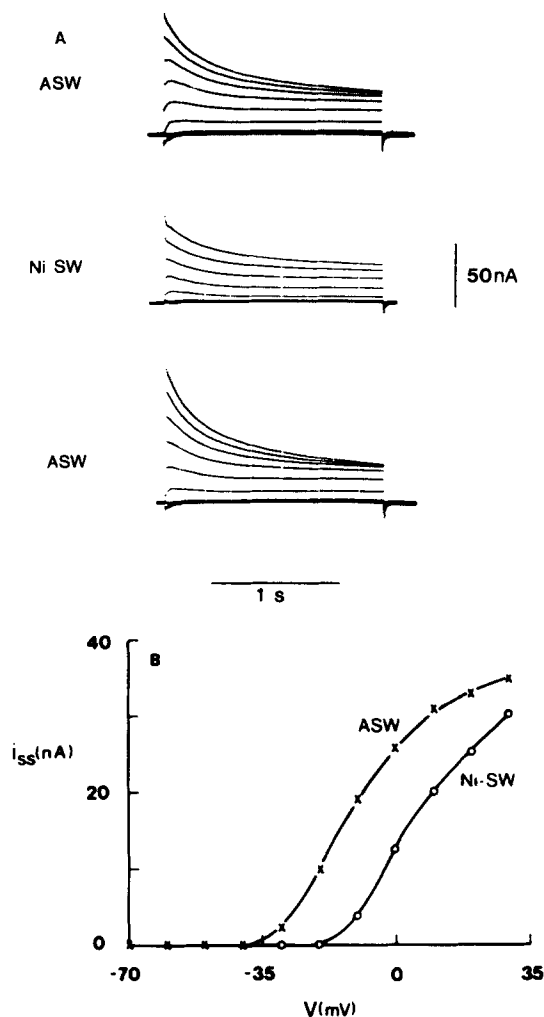


FIGURE 9. Effects of extracellular  $\text{Ni}^{2+}$  on  $i_A$  and  $i_{88}$ . (A)  $\text{Ni}^{2+}$  reduced both maintained and transient outward currents elicited by voltage-clamp pulses from  $V_H = -70$  mV. Currents are from voltage pulses in 10-mV increments from  $-60$  to  $+30$  mV. The effect of  $\text{Ni}^{2+}$  was partially reversible. (B) The voltage dependence of  $i_{88}$  is shown for ASW ( $\times$ ) and NiSW ( $\circ$ ). (C) The voltage dependence of  $i_A$  is shown for ASW ( $\times$ ) and NiSW ( $\circ$ ).

$\text{Ni}^{2+}$  may also produce a small reduction of  $\bar{g}_K$ , as has been reported previously in *Xenopus* myelinated axons (Arhem, 1980).

Since the evidence based on pharmacological block of inward  $\text{Ca}^{2+}$  current is inconclusive, other lines of investigation that bear on the existence of  $\text{Ca}^{2+}$ -

activated  $K^+$  channels must be considered. First, the results of Pepose and Lisman (1978) show that outward currents are not activated by intracellular injection of  $Ca^{2+}$ . Second, we show here that intracellular injection of EGTA sufficient to stabilize the light-activated currents (Fig. 10) reduces the voltage-dependent outward currents by only 25%. Third, removing  $Ca^{2+}$  from the extracellular medium does not significantly reduce outward currents (Fig. 8). It might be argued that it is impossible to lower extracellular  $Ca^{2+}$  significantly because of the highly invaginated membrane of the ventral photoreceptor. This seems unlikely, however, because removal of  $Ca_o$  blocks both the residual voltage-dependent inward current in 0-Na seawater (Fig. 2) and the desensitization of the light response produced by depolarization (O'Day et al., 1982). Taking these results together, it is very unlikely that a large fraction of maintained outward current is carried by a  $Ca^{2+}$ -activated  $K^+$  channel,

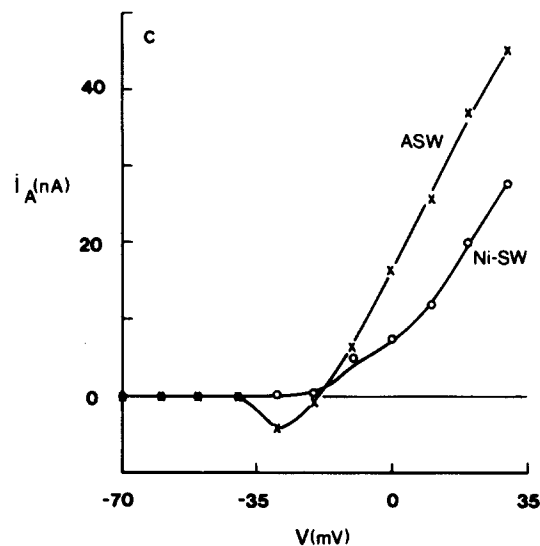


FIGURE 9.

although the possibility that a small fraction of outward current is carried by such a channel cannot be excluded.

#### CONCLUSION

The membrane of *Limulus* ventral photoreceptors contains at least three separate voltage-dependent conductances. These conductances are activated by depolarization from resting potential, and consequently must be activated during at least part of the normal light response. Much remains to be understood about the kinetics of these conductances before their separate contributions to the receptor potential can be predicted mathematically. In particular, it will be necessary to discover some means of completely separating inward from outward currents (as, for example, in Akaike et al., 1978). However, we have found here that each of the conductances can be blocked

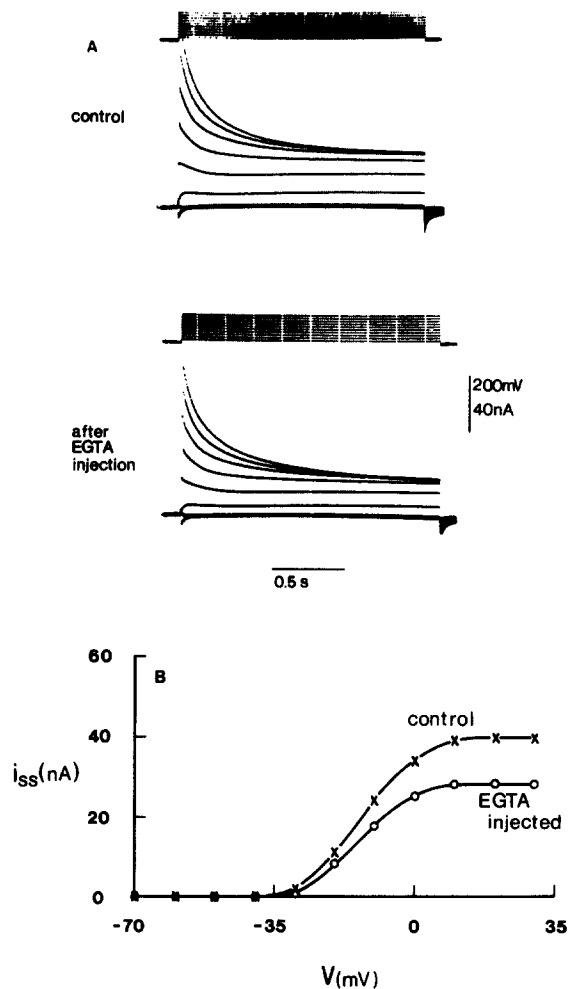


FIGURE 10. Effects of intracellular EGTA injection on voltage-dependent and light-induced currents. (A) EGTA injection reduced somewhat (30% at +30 mV) the maintained current,  $i_{ss}$ , elicited by voltage-clamp pulses ( $V_H = -70$  mV).  $i_A$  was less affected. (B) The voltage dependence of  $i_{ss}$  is shown for control (x) and EGTA-injected (o) conditions. (C) The voltage dependence of  $i_A$  is shown for control (x) and EGTA-injected (o) conditions. (D) EGTA injection was sufficient to alter the light response in this cell. The cell was voltage-clamped to  $-70$  mV in the dark. A prolonged bright light ( $4 \times 10^{-5}$  W/cm<sup>2</sup>, 530 nm) was presented to the photoreceptor. The light-induced currents exhibited a characteristic transient phase followed by a plateau phase that persisted until the end of illumination. After EGTA injection the rise time of the light response was much longer, and the transient and plateau phases were indistinguishable. The total EGTA injected was 25 mM, assuming a transport coefficient of 0.5 and a cell volume of  $5 \times 10^{-10}$  liter.

pharmacologically in a relatively specific manner. Thus, it is possible to study the role each conductance plays in shaping the receptor potential using pharmacological methods. The results of such a study are presented in the following paper (O'Day et al., 1982).

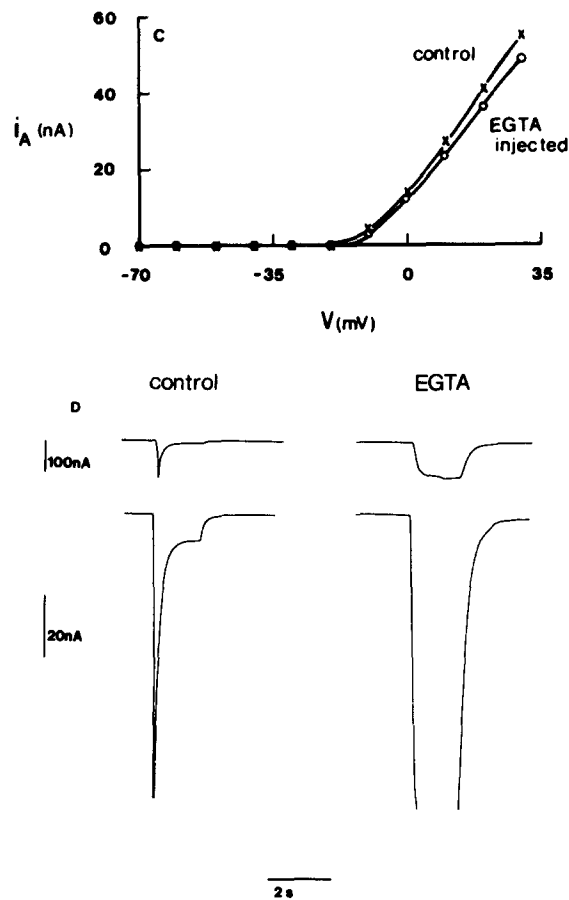


FIGURE 10.

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